INFLAMMATORY BIOMARKERS AND ITS VALUE IN PREDICTING SURVIVAL AND OUTCOME AMONG PATIENTS WITH SPONTANEOUS INTRACEREBRAL HAEMORRHAGE

by

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LIST OF ABBREVIATIONS

1)	GCS	-	Glasgow Coma Scale
2)	WBC	-	White Blood Cell
3)	CRP	-	C – Reactive Protein
4)	SICH	-	Spontaneous IntraCerebral Haemorrhage
5)	GOS	-	Glasgow Outcome Scale
6)	EDTA	-	EthyleneDdiamineTetraacetic Acid
7)	Cum. (in Kaplan- Meier curve)	-	Cumulative
8)	Q1	-	1^{st} Quartile (25^{th} percentile)
9)	Q2	-	2^{nd} Quartile (50^{th} percentile)
10)	Q3	-	3^{rd} Quartile (75^{th} percentile)
11)	Q4	-	4^{th} Quartile (above 75^{th} percentile)
12)	SD	-	Standard Deviation
13)	N	-	Total Number
14)	PMN	-	Polymorphonuclear
15)	IL-1	-	Interleukin-1
16)	IL-6	-	Interleukin-6
17)	CT scan brain	-	Computed Tomography of brain

ABSTRAK

Latar Belakang

Kebelakangan ini, penyakit pendarahan otak secara spontan telah muncul sebagai salah satu daripada jenis penyakit strok atau angin ahmar yang paling dashyat dan memudaratkan. Penyakit ini mampu menyebabkan maut sebanyak 45% dalam 30 hari selepas pendarahan otak. Lebih banyak penyiasatan makmal and bukti klinikal yang dijalankan dalam tiga dan empat dekad yang lalu telah membuktikan bahawa mekanisme keradangan atau ‘inflamasi’ yang kompleks bertangungjawab untuk kesan mudarat yang dialami pesakit yang mengidapi penyakit pendarahan otak secara spontan. Penanda-penanda biologi dan sel-sel yang terlibat dalam proses keradangan ini memainkan peranan penting dalam propogasi penyakit dan kesan kemudaratan kepada pesakit. Walau bagaimanapun, hubungan di antara penanda-penanda biologi dalam darah dengan kesan kemudaratan dalam pesakit-pesakit ini tidak dapat dikenalpasti dengan lebih terperinci and objektif. Hubungan diantara penanda-penanda biologi dan hasil pemulihan dan fungsi pesakit-pesakit ini tidak dapat ditentukan dengan kepastian. Justeru itu, penyelidikan ini bertujuan untuk memberi satu rangka dan bukti untuk mengaitkan paras penanda-penanda biologi seperti sel darah putih dan C-Reactive Protein (CRP) dalam penyakit pendarahan otak dan sekaligus mengkaji hubungan penanda-penanda biologi ini dengan tahap kemandirian pesakit pada 6 bulan selepas mengalami pendarahan otak. Penyelidikan ini juga bertujuan untuk membuktikan bahawa paras sel darah putih and CRP mampu digunakan sebagai satu alat untuk menentukan kadar fungsi atau kemudaratan dan kematian dalam pesakit-pesakit yang mnegalami pendarahan otak secara spontan.

Kaedah Penyelidikan

Penyelidikan ini dijalankan secara prospektif dalam tatacara kerelasi untuk mangkaji kaitan di antara paras sel darah putih dan paras CRP pada waktu kemasukan hospital dan pada jangka masa 72 jam selepas kemasukan ke hospital, dengan tahap kemandirian dan kadar kematian pesakit pada 6 bulan selepas mengalami pendarahan otak. Kajian ini dijalankan di Hospital Umum Sarawak (HUS) dimana terdapat perkhidmatan neurosurgeri dan perubatan neurologi. Kajian ini dijalankan selama 2 tahun , di antara April 2013 ke April 2015. Butir-Butir lain seperti umur pesakit, jantina, skor Glasgow Coma Scale (GCS), tekanan darah semasa kemasukan hospital and butiran mengenai penyakit-penyakit tidak berjangkit yang dihidapi oleh pesakit diambil and dicatat. Sampel darah diambil sebanyak 2 kali – iaitu semasa kemasukan pesakit ke hospital dan sekali lagi dalam pada 72 jam selepas kemasukan ke dalam wad. Setiap pesakit juga menjalani penyiasatan radiologi dan pengimejan dalam bentuk CT scan otak untuk mengenalpasti ciri-ciri berkaitan pendarahan otak ; yakni berkaitan lokasi pendarahan otak serta jumlah isipadu pendarahan tersebut. Pesakit-pesakit yang layak dipilih untuk mengambil bahagian dalam kajian ini setelah memenuhi semua syarat-syarat yang ditetapkan. Data yang diperolehi akan dianalisakan dengan mengunakan sistem perisian "Statistical Package for Social Sciences (SPSS) for Windows" versi 21.0. Setelah peaskit menjalani rawatan dan dibenarkan pulang ke rumah, mereka akan disaring dan diperiksa semula di Klinik Neurosurgeri semasa lawatan susulan. Skor 'Glasgow Outcome Score (GOS)' ditentukan kelak semasa lawatan susulan pesakit-pesakit ini.

Keputusan

Seramai 60 orang pesakit dipilih untuk mengambil bahagian dalam kajian ini. Jumlah pesakit yang terdiri daripada kumpula pesakit dengan tahap kemandirian yang memuaskan atau baik (GOS 4-5) adalah seramai 20 orang (33.3%) dan jumlah pesakit yang mengalmai tahap kemandirian yang buruk atau tidak memuaskan adalah seramai 27 orang (45%). Seramai 13 orang pesakit meninggal dunia (GOS 1) dalam masa diantara kemasukan hospital dan 72 jam selepas kemasukan hospital. Daripada jumlah 60 ornang pesakit ini, kita mendapati seramai 16 orang pesakit (26.7%) terdiri daripada golongan umur pada atau kurang daripada 50 tahun, dan seramai 44 orang pesakit (73.3% terdiri daripada golongan yang lebih tua daripada umur 50 tahun. Kebanyakan pesakit didapati dimasukkan ke hospital dengan skor GCS 9/15 ke 11/15 iaitu seramai 13 orang (21.7%) untuk setiap skor GCS 9/15, 10/15, 11/15. Seramai 5 pesakit (8.3%) sahaja didapati mempunyai skor GCS 14/15 semasa kemasukan hospital. Dalam kajian ini, kami mendapati bahawa skor GCS semasa kemasukan hospital berkait rapat dengan tahap kemandirian dan kadar kematian pesakit pada 6 bulan selepas mengalami pendarahan otak ($p<0.05$). Jumlah isipadu dan saiz pendarahan otak juga berhubung rapat dan secara langsung mempengaruhi tahap kemandirian dan kadar kematian pada masa 6 bulan selepas pendarahan otak ($p<0.05$). Paras sel darah putih juga berhubung rapat dengan saiz dan isipadu pendarahan otak, yakni, pesakit yang mengalami pendarahan otak yang besar, mempunyai paras sel darah putih yang tinggi. Ciri-ciri seperti umur dan jantina didapati tidak berkaitan secara langsung dengan tahap kemandirian atau dengan kadar kematian pesakit-pesakit ini pada 6 bulan selepas pendarahan otak. Didapati juga paras sel darah putih dan CRP semasa kemasukan hospital dan pada 72 jam selepas kemasukan hospital mempunyai hubungan yang langsung dan jelas dalam

meramalkan tahap kemandirian dan kadar kematian pesakit-pesakit ini6 bulan selepas mengalami pendarahan otak ($p<0.05$)

Kesimpulan

Secara kesimpulannya, kami mendapati bahawa unsur-unsur seperti skor GCS semasa kemasukan hospital, saiz pendarahan otak, paras sel darah putih dan CRP pada waktu kemasukan ke hospital dan pada 72 jam selepas kemasukan hospital, secara langsung dapat meramalkan tahap kemandirian dan kadar kematian pesakit-pesakit yang mengalami pendarahan otak secara spontan.

ABSTRACT

Background

Spontaneous IntraCerebral Hemorrhage (SICH) has emerged as one of the most devastating forms of stroke in recent decades. This disease is noted to carry a 30 day mortality rate of approximately 45%. An increasing number of studies have implicated a complex immune-mediated and inflammation mediated cascade of responses in the pathophysiology of SICH and the resultant neurologic outcome. Several clinical studies have demonstrated an association between inflammatory markers and outcome in patients with SICH. However, the exact relationship between serum biomarkers and functional outcomes amongst survivors has not been clearly elucidated . This study aims at providing a promising perspective and to evaluate the changes in peripheral leukocyte count (WBC count) and C-Reactive Protein (CRP) level in patients with SICH and to correlate these findings with survival and functional outcome, thus to to support and substantiate existing evidences.

Methodology

A prospective, descriptive and correlational study was conducted in Hospital Umum Sarawak (HUS) over the span of 2 years (April 2013 till April 2015) . Patients with supratentorial intracerebral bleed secondary to uncontrolled hypertension, aged between 30-75 years were recruited in this study . Data pertaining to the demography (age, gender, BP, GCS score, and

co-morbidities) , clinical and radiological parameters (site of lesion and the volume of the clot) were collected on admission. Blood samples were taken to measure peripheral WBC count and CRP level on admission and at 72 hours of admission. Mortality and functional outcomes were determined at 6 months post ictus. Patients were recruited following fulfillment of exclusion and inclusion criteria and all obtained data was analyzed with Statistical Package for Social Sciences (SPSS) for Windows version 21.0.

Results

A total of 60 patients were recruited in this study. We found about 16 patients were less than or equal to 50 years old (26.7%) and 44 patients belong to the older age group of above 50 years (73.3%). Majority of patients presented with GCS score of 9/15 to 11/15 with a total of 13 patients (21.7%) in each group of 9/15, 10/15 and 11/15. The least number of patients encountered belonged to the GCS score of 14/15 – a total of 5 patients (8.3%). GCS score on admission was noted to be significantly related to 6 month functional outcome or Glasgow Outcome Scale (GOS) and overall mortality or survival ($p<0.05$). The morbidity and overall mortality or survival was also found to be significantly associated with volume of clot at presentation ($p<0.05$). WBC count was also noted to be correlating with haematoma volume at admission – patients with large haematoma volume inevitably had elevated WBC count. Age and gender was not found to be significantly related to the survival or 6 months functional outcome ($p>0.05$). WBC count and CRP level on admission and at 72 hours of admission noted to have relationship with overall 6 months GOS or functionality and also with overall survival ($p<0.05$).

Total number of patients belonging to the better Glasgow Outcome Score (GOS) group (GOS 4-5) was found to comprise 20 patients (33.3%) and about 27 patients (45%) belonged to the poor GOS group (GOS 2-3) . About 13 patients (21.7%) succumbed to the disease (GOS 1).

Conclusion

We could conclude that via this study, it was evident that in patients with SICH, the main determinants or predictors of functional outcome at 6 months and overall survival were noted to be GCS score on admission, clot size, WBC count and CRP levels on admission and at 72 hours of admission.

CHAPTER 1

Introduction

Spontaneous IntraCerebral Hemorrhage (SICH) has emerged as one of the most devastating subtype of stroke. Epidemiologically, SICH is a leading cause of morbidity and mortality worldwide (Broderick JP et al., 2007). It has been proven to pose worse outcomes than ischemic stroke (Gere J et al., 2003), and is increasing in prevalence currently owing to the advancement in primary care – both in clinically and radiologically ; as more and more of these cases are detected and treated early . It has a 30 day mortality of 40-50% (Roger VL et al., 2011; Van Asch CJ et al., 2010). Despite the knowledge of the pathophysiology and acute treatment of stroke has advanced by leaps and bounds in the past decade, progress observed with SICH has been much slow-paced and phlegmatic (Mayer and Rincon, 2005; Qureshi et al., 2009). The burden of a long term disability and poor outcome in SICH are still deemed high, with only < 40% of patients becoming independent at 1 year (Van Asch CJ et al., 2010) despite the fact that there has been advances in management and clinical knowledge in recent times.

Causative factors of SICH are classified into as either primary – which are causes unrelated to congenital or acquired lesions) or secondary – which are attributed to congenital or acquired lesions. Primary SICH accounts for 78% to 88% of cases and originates from the spontaneous rupture of small arteries or arterioles damaged by two major causes: hypertensive arteriolosclerosis and amyloid angiopathy (Mayer and Rincon, 2005; Qureshi et al., 2001; Sutherland and Auer, 2006). Ariesen MJ et al., 2003 identified hypertension as the most significant modifiable risk factor, occurring in 50-70% of patients. On the other hand, secondary

SICH is caused by identifiable lesions in brain such as tumour, aneurysms and vascular anomalies. Secondary SICH is also increasingly found to be related to the use of anti-coagulant or coagulopathic states, and thrombolytic treatment of ischemic stroke (Mayer SA et al., 2005; Qureshi AI et al., 2001; Sutherland GR et al., 2006 ; Wang J et al., 2005). In all SICH cases, the extravasated blood accumulates and compresses the surrounding brain parenchyma tissue. The commonest location of SICH are ganglionic (putamen, caudate nucleus and thalamus), followed by lobar, cerebellar and pontine or brainstem. In this study, we have only examined and recruited patients presenting with primary supratentorial SICH (bleeding in the region of putamen, caudate nucleus or cortical lobar) due to hypertension. Mainstay goal of treatment for SICH is merely supportive, and the anticipated long term clinical outcome is generally poor and the potential extensive burden for the primary caretakers is ubiquitous.

It is imperative at this juncture that we would like to stress that to improve the clinical outcome of patient with SICH, a sound understanding of the pathophysiology of SICH induced brain injury is of paramount importance. Recently there has been more than compelling evidences to suggest that robust inflammatory mechanisms are triggered in SICH induced brain injury (Aronowski J et al.,2005 ; Wang J et al.,2005).

Loftspring MC et al. in 2010 , found that in animal models, a robust inflammatory response is activated by the entry of blood into the brain parenchyma with a consequent infiltration of peripheral leukocyte or White Blood Cells (WBC), activation of microglia and release of cytokines. This findings were further reciprocated in other studies (Xi G et al.,2006 ; Wang J et al.,2007). Autopsies conducted in both animals (Gong C et al.,2000) and in human subjects (Mackenzie JM et al.,1998) with SICH found leukocytic infiltration usually occurs within the

first 3 days of insult and a further cascadial inflammatory changes in the penumbra of the hemorrhage following SICH comes into play in the natural history of the disease. In the past, very few clinical studies have demonstrated an association between the inflammatory markers and outcomes of SICH in terms of mortality (Di Napoli M et al.,2011). Another inflammatory biomarker that has sparked a recent interest in the research of neuro-inflammation related to SICH is C-Reactive Protein (CRP). Di Napoli M et al. in 2011 discovered that elevated levels of CRP, an acute-phase reactant induced by IL-6, is directly associated with 30-day mortality in SICH patients.

To our knowledge, very few studies evaluated the functional or long term outcomes in SICH in relation to WBC and CRP levels. Several prospective studies in ischemic stroke patients have reported that increased levels of acute inflammatory markers, such C-reactive protein (CRP) (Jennifer G et al.,2009 ; Di Napoli M et al.,2011) and white blood cell (WBC) count (Wang J et al.,2007) are associated with increased risk of death or disability. The prognostic role of these inflammatory markers after SICH is ambiguous (Diamond P et al.,2003 ; Flaherty ML et al.,2006). In this study, we have evaluated the relationship between activation of the inflammatory response as measured by change in peripheral WBC count, and CRP levels – to mortality and functional outcomes after SICH.

Currently existing data that was collected from both preclinical and clinical studies illustrates that inflammatory processes are involved in SICH (Aronowski J et al., 2005) and in the evolution of SICH induced brain injury (Wang J et al. 2007). Several prospective studies have been conducted implicated that brain injury secondary to a SICH is characterized by acute

local inflammation (Wang J et al.,2007 ; Keep RF et al.,2005) and the elevated levels of inflammatory markers after SICH predict worse prognosis (Castellanos M et al.,2005) , perihematoma brain oedema (Castillo J et al.,2002), early neurological deterioration (Leira R et al.,2004), and early growth of SICH (Silva Y et al., 2005). Elevated WBC count has been proclaimed to be one of the independent predictors of early neurologic deterioration in SICH (Silva Y et al.,2005 ; Leira R et al.,2004) . As stressed earlier, CRP , another promising inflammatory biomarker, has been postulated to predict prognosis in patients presenting with SICH (Di Napoli M et al.,2005). These findings has catalyzed renewed interest in the study of neuro-inflammation related to SICH which includes the study of relation of SICH prognosis and outcome to CRP level and WBC count. Therefore, in this study the potency of WBC and CRP in determining the eventual outcome and mortality in SICH patients are investigated. The understanding of physiology of WBC and CRP is important before the realm of neuro-inflammation related to SICH is explored and studied.

White blood cells (WBCs), also called leukocytes , are the 'mobile units' of the immune system responsible in protecting the body against infectious disease. It is the first line defense against non-bodily foreign invaders and catalyzes the chain of events that brings about inflammation. All leukocytes are produced and derived from a pluripotential haematopoietic stem cell in the bone marrow. There are 6 different and diverse types of leukocytes identified – namely the **neutrophils, eosinophils, basophils, lymphocytes, monocytes and occasional plasma cells** (Ford-Hutchinson A et al.,1985) These types are distinguished by their physical and functional characteristics. The number of leukocytes in the blood is often a reliable indicator

of disease (Feuerstein G et al.,1987) The normal WBC count is usually between 4 and 11 × 10^9/L and they make up approximately 1% of the total blood volume in an adult.

Neutrophils constitute the most in total WBC count which is about 60-70% of the circulating leukocytes (Bruce Alberts et al.,2002) . Neutrophils defend against bacterial or fungal infection and are usually first responders to microbial infection. They are commonly referred to as polymorphonuclear (PMN) leukocytes. They have a multi-lobed nucleus, which consists of three to five lobes connected by slender strands (Saladin and Kenneth 2012) and this gives the neutrophils the appearance of having multiple nuclei, hence the name polymorphonuclear leukocyte. Neutrophils are mature cells as compared to monocytes, that can attack and destroy bacteria even in circulating blood. Average life span of a circulating human neutrophil is about 5.4 days (Pillay J et al 2010).

Eosinophils comprise about 2-3% of the total WBC. Eosinophils are weak phagocytes and their counts fluctuates throughout the day, seasonally, and during menstruation. Eosinophils have a propensity to rise in response to allergies, parasitic infections, collagen diseases, and disease of the spleen and central nervous system. They are found in abundance in the mucous membranes of the respiratory, digestive, and lower urinary tracts (Saladin and Kenneth 2012). They primarily deal with parasitic infections and are also the key role player as the predominant inflammatory cells in allergic reactions. The most important causes of eosinophilia include allergies such as asthma, hay fever, and hives; and also parasitic infections. Although most of the parasites are too large to be phagocytized by eosinophils, they secrete chemicals that destroy

these large parasites, such as hook worms and tapeworms, that are too big for any one WBC to phagocytize (Howard J etal.,1984)

Basophils are chiefly responsible for allergic and antigen response. Because they are the rarest of the white blood cells (less than 0.5% of the total count) and share physico- chemical properties with other blood cells, they are difficult to study (Falcone and Franco 2000) They excrete two chemicals that aid in the body's defenses: histamine and heparin. Histamine plays an exceedingly important role for widening blood vessels which in turn increases the flow of blood to injured tissue. It also promotes increased permeability of blood vessels to enable neutrophils and clotting proteins to get into connective tissue more easily. Heparin is an anticoagulant that inhibits blood clotting and promotes the movement of white blood cells into an area. Basophils can also release chemical signals that attract eosinophils and neutrophils to an infection site.

Lymphocytes are much more common in the lymphatic system than in blood. They play a pivotal role in body’s acquired immunity (Beer J et al.,1984). Lymphocytes include:

- **B cells** – provides body with humoral immunity which make antibodies that can bind to pathogens, block pathogen invasion, activate the complement system, and enhance pathogen destruction.
- **T cells** – provides body with cell mediated immunity. Several types identified :
    - CD4+ helper T cells
    - CD8+ cytotoxic T cells:
    - γδ T cells

- Natural killer cells - role is implicated in destruction of cells of the body that do not display MHC class I molecules, or display stress markers such as MHC class I polypeptide-related sequence A (MIC-A). A decreased expression of MHC class I and up-regulation of MIC-A can happen when cells are infected by a virus or become cancerous (Heberman R et al.,1987)

Monocytes share the phagocytosis function of neutrophils, but are much longer lived as they have an extra role: they present pieces of pathogens to T cells so that the pathogens may be recognized again and killed. This causes an antibody response to be mounted. Monocytes eventually leave the bloodstream and differentiate into mobile tissue macrophages

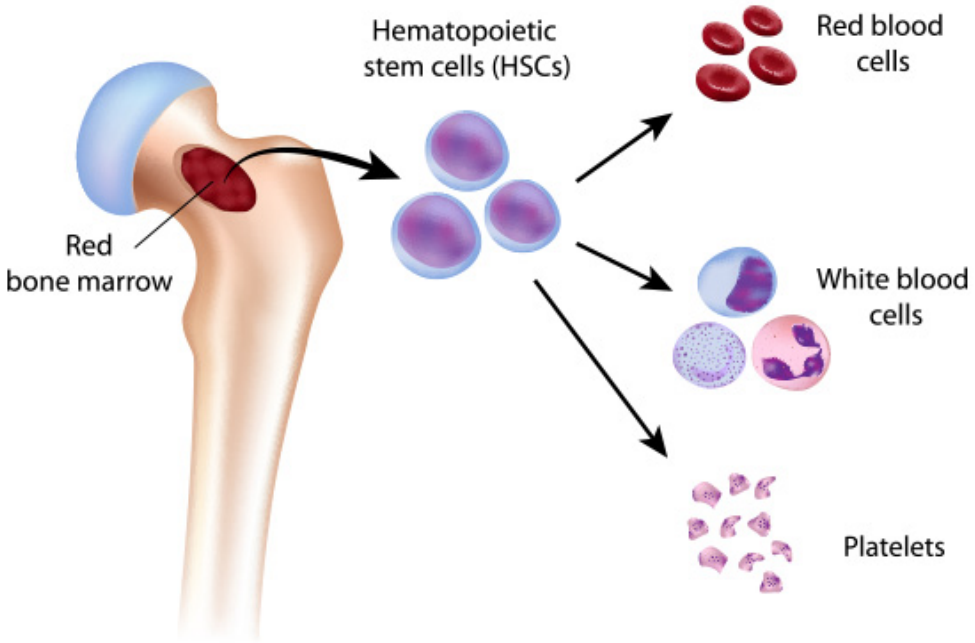


Figure 1 : Diagram showing the source of WBC

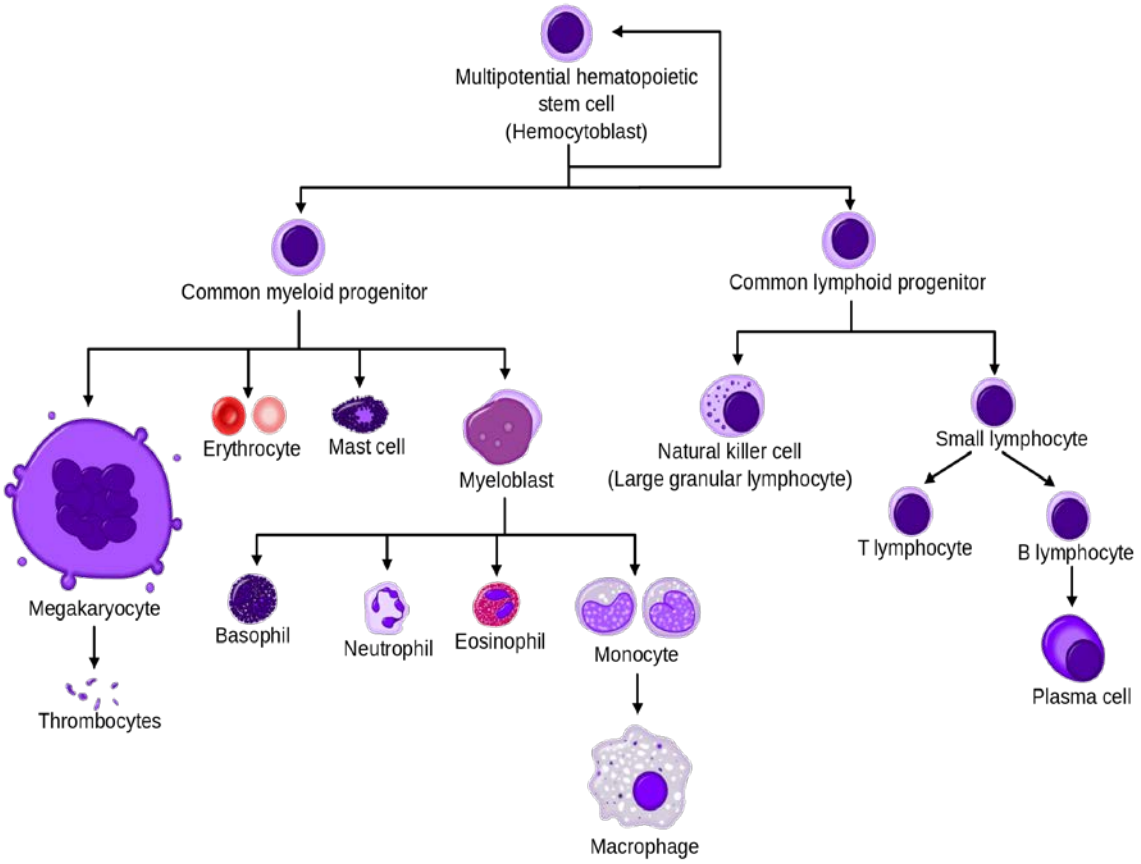


Figure 2 ： Diagram illustrating the formation and differentiation of WBC

Another inflammatory biomarker implicated in this study is CRP. C- reactive protein (CRP) was discovered by *Tillett* and *Francis* in 1930 (Tillet WS et al. 1930). They first discovered it as a substance in the serum of patients with acute inflammation that reacted with the C- (capsular) polysaccharide of pneumococcus (Tillet WS et al 1930) . CRP was initially postulated as a pathogenic secretion as it was elevated in people with a variety of illnesses including cancer (Pepys et al. 2003). However, the discovery of the hepatic synthesis of CRP by Peter J Kennelly et al. in 2007 demonstrated that it is a native protein. This finding was further reinforced by research conducted by Matthew R et al. in 2007. CRP is produced mostly by liver

hepatocytes in response to cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNF) (Butterweck V et al.,2003) which are triggered in acute inflammation.

CRP is a highly conserved trace protein found in the circulation of even healthy subjects, with a median concentration of approximately 1 mg/L(<5mg/L) . As the quintessential member of the acute phase proteins its concentration could increase upto 100-fold or more in response to injury, infection, or inflammation (Erren M et al.,1999). Sometimes, acute phase phenomena may also accompany chronic inflammatory disorders. Moderately increased plasma CRP concentrations are found in smokers and under conditions of atherosclerosis, hypertension, psychological stress, diabetes, and in the elderly (Cao JJ et al.,2003) These levels are however not significantly elevated . Any levels above 5mg/L should raise the suspicion of an ongoing or underlying inflammatory process (Pepys MB et al.,2003). As CRP is a molecule that acts via pattern recognition , it binds to specific molecular configurations that are typically exposed during cell death or found on the surfaces of pathogens. John J et al. in 2008 concluded that rapid increase in CRP levels within hours following tissue injury or infection suggests that it contributes to host defense or innate immune response. CRP is also found to be able to interact with DNA and histones and in turn may scavenge nuclear material released from damaged circulating cells (Wolford JK et al. 2003).

The CRP cytogenetic band is located on the first chromosome - 1q21-q23. Wolford JK et al. in 2003 also discovered that it has a 224-residue protein with a monomer molar mass of 25106 Da and the assumes an annular pentameric disc shape.

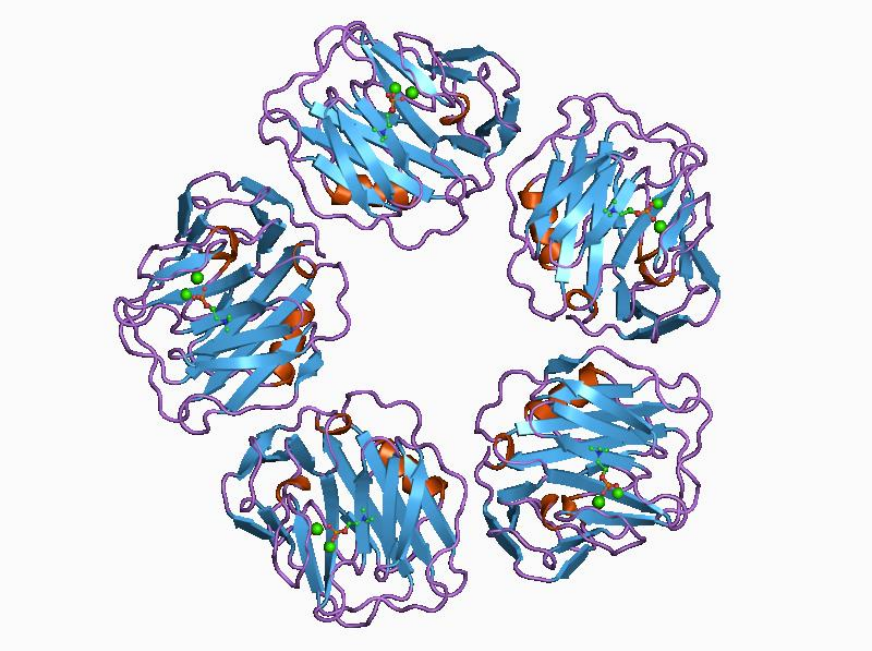


Figure 3 Pentameric structure of CRP viewed down the 5-fold symmetry axis.

CRP assay and analysis could be done via various analytical methods. One such method is done by collecting the blood in a serum-separating tube and is analyzed in a medical laboratory via ELISA (Enzyme-Linked Immunosorbent Assay). This technique essentially requires any ligating reagent that can be immobilized on the solid phase along with a detection reagent that will bind specifically and use an enzyme to generate a signal that can be properly quantified (Thompson D et al.,1999).

Conventionally, there are two different tests for CRP. The standard CRP test determines CRP levels over a much wider range and is deemed less sensitive in the lower ranges. On the other hand, the high-sensitivity CRP (hs-CRP) test can more accurately and rigorously detect lower concentrations of the protein. It is proven that estimating the levels of hs-CRP is more

useful than the standard CRP test in predicting a healthy person's risk for cardiovascular related diseases (Clyne B et al.,1999). In this study, we have measured the standard CRP levels in patients as the cost of measuring the levels of hs-CRP is not economically feasible.

CHAPTER 2

LITERATURE REVIEW

2.1 Background

Rosamond W et al. in 2007 concluded that Spontaneous IntraCerebral Haemorrhage (SICH) accounts for 8% to 14% of all strokes and carries the highest mortality rate among the major stroke subtypes . Current available pharmacological and surgical interventions are less efficient than in case of ischaemic stroke (Steiner T et al.,2014). Approximately half of the mortality occurs within the first 2 days as a result of brain herniation (Broderick JP et al.,1999). SICH is estimated to occur in about 70,000 people a year in the United States (Morgenstern LB et al.,2010). It accounts for 20-30% of all type of stokes in Asian countries (Quereshi AI et al.,2009). SICH was noted to be occurring more common in men, in elderly people and of Asian and African American descent (Sacco RL et al.,1994).

The most important risk factor for ICH is identified as age. A 2 fold increase in incidence is observed in aach advancing decade from 50-80 years. The incidence of SICH increases with age and the average risk doubles for every 10 years after the age of 35 (Broderick et al.,1999). Other identifiable significant risk factor is hypertension is the most significant modifiable risk factor, occurring in 50-70% of patients (Arlesen MJ et al.,2003). Non modifiable risk factors were gender (male sex) and age. Other modifiable risk factors attributed to SICH are diabetes mellitus (Juvela et al.,1996), smoking (Thrift et al.,1998), dyslipidaemia (Sturgeon et al.,2007) and large quantity of alcohol intake (Ariesen et al.,2003). Hypertension is mainly associated with deep cerebral, ganglionic, lobar, cerebellum and brainstem locations (Flaherty ML et al.,2006)

Hypertension was identified as most prevalent modifiable risk factor in SICH accounting for about 60-70% of all cases (Brott et al.,1986) . The presence of apolipoprotein E2 and E4 alleles in patients was also found to be related to increase in incidence of vascular amyloid deposition which in turn may result in lobar SICH (Woo et al.,2005).

The identified common causes of SICH was hypertension which accounts for about 60-70% of the cases (Brott et al.,1986). Chronic hypertension causes degeneration, fragmentation and fibrinoid necrosis of small perforating arteries which would be later prone for rupture in case of elevated BP (Fischer et al.,1971), Cerebral amyloid angiopathy accounts for the 2nd most common cause for SICH (Qureshi AI et al.,2001). Other secondary causes of SICH include vascular malformations, tumour, haemorrhagic transformation of infarct, anti-coagulants, and cerebral venous thrombosis (Manno et al.2005)

2.2 Pathophysiology of SICH and the role of neuro-inflammation

The primary brain injury after SICH is mainly caused by the complex dynamic formation of the blood clot which damages and disrupts the surrounding tissue. This causes a mechanical damage associated with the clot mass and it in turn will exert a mass effect.damage associated with the mass effect (Xi G et al.,2010). Besides treating increased intracranial pressure (Helboketal et al., 2011), surgical interventions to remove the blood clot and immediately decompress the mounting pressure is the mainstay of treatment. (Gautschi and Schaller et al.,2013). However, in about one third of patients (Kazuietal et al.,1996; Brottetal et al.,1997), re-bleeding may occur and resulting in the expansion of the hemorrhage within the first day following insult. This in turn will further worsened the mass effect and thus causing further

deterioration of neurological status. Main strategy in preventing this complication is by aggressive antihypertensive therapy or by administration of hemostatic factors may prevent secondary hematoma growth.(Sakamoto et al.,2013).. Breakdown of blood brain barrier is also attributed to early expansion of SICH (Olson et al.,1993) .

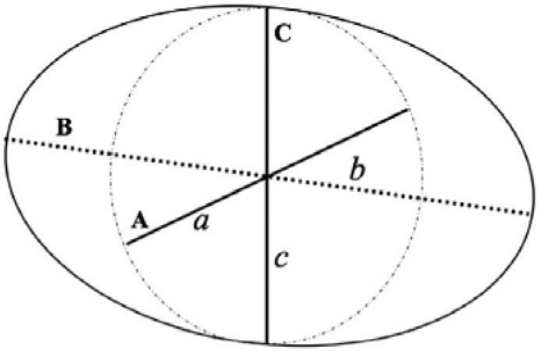
Secondary brain injury after SICH may be caused by a cascade of events initiated by the primary injury which triggers the body/tissue response to the haematoma or the aseptic inflammation. The release of clot components like haemoglobin and iron may trigger a multi-tiered cascade of inflammatory responses and liberation of inflammatory and chemical mediators . Thrombin release is an important initial tissue response to SICH which activates the haemostatic mechanisms to curb the initial bleeding. Thrombin plays a central role in that haemostasis. However, there is also much evidence that thrombin can participate in ICH-induced injury. Thrombin formation may cause neuro-toxicity and cause inflammatory cell infiltration, mesenchymal cell proliferation, scar formation, brain oedema formation and seizures., leading to disruption of blood-brain-barrier (Xi G et al.,2003). Liberated thrombin also is found to induce apoptosis in cultured neurons
and astrocytes (Donovan FM et al.,1997) . Liu DZ et al. in 2011 found that thrombin also contributes to mitogenic stress, excitoxicity, vascular hyperpermeability and inflammation. Studies have shown that thrombin inhibition can reduce ICH induced injury (Xi G et al. 2003). However, it is imperative to highlight that while high concentrations of thrombin may mediate ICH-induced brain injury, low concentrations are neuroprotective.(Vaughan PJ et al.,1995) and is involved in brain recovery and neurogenesis (Yang S et al.,2008)

More and more clinical trials have compiled evidences that suggest inflammatory mechanisms are involved in the progression of ICH induced brain injury. These inflammation was mediated by molecular components like progtaglandins, chemokines, cytokines (Tumour necrosis factor (TNF), Interleukins (IL) 1 and 6), complement system, extracellular proteases and reactive oxygen species (Wang J et al.,2007). The are also cellular mediated response induced injury brought upon by leukocytes and microglia.

2.3 Measurement of ICB volume

It is exceedingly evident now that volume of intracerebral hemorrhage is the strongest predictor of 30-day outcome for all locations of spontaneous intracerebral hemorrhage (Hanel et al.,2002). Commonly used method for estimation of volume of the clot is via Kothari's method (AxBxC/2) (Kothari RU et al.,1996). For the purpose of prognosis, a model of 30-day mortality that used the Glasgow Coma Scale (GCS) score and hemorrhage volume in patients with ICH correctly predicted outcome with a sensitivity and specificity of 97% (Broderick JP etal.,2003). This method of estimating ICH volume was even used in the for patient eligibility assessment in the multicenter Surgical Trial of IntraCerebral Hemorrhage (STICH trial) (J. Grotta et al. 1996)

Figure 4 : The estimation of volume for an ellipsoid object is 4/3 x π x A x B x C



$$V = \frac{\pi \times 4 \times a \times b \times c}{3} = 4 \times \frac{A}{2} \times \frac{B}{2} \times \frac{C}{2} = \frac{A \times B \times C}{2}$$

Figure 5 : Calculation of the volume of the haematoma based on the volumetric formula for an ellipse. In this figure a,b, and c are the radius of the sphere; A,B and C are the diameters of the sphere; and π: 3.142

CHAPTER 3

STUDY PROCEDURE

3.1 Problem statement

In recent years, it is been increasingly recognized that pathophysiology involved in spontaneous ICH is related to the patient's immune response to the insult. As illustrated in the study conducted by Wang J et al. in 2010, the infiltrating white blood cells (WBC) play a role in secondary injury after ICH. Preclinical studies have proven that leukocytes are present surrounding the area of hematoma within hours after ICH (Zhao X et al.,2006). These neutrophils infiltrate the ICH first and may in turn catalyze a cascade of immune responses and cause direct detrimental effect or injury by releasing reactive oxygen species, inflammatory proteases and damage to the blood brain barrier.(Wang J et al.,2010 ; Dore S et al.,2007). Recently, elevated levels of CRP has also been implicated in poorer outcome in patients with SICH and plays a significant role in prediction of their prognosis (Godoy DA et al.,2010). Despite all available evidences, very few studies have examined the efficacy of WBC and CRP levels exclusively, as a tool to predict long term outcome and survival. If elevated peripheral leukocytes and other available inflammatory biomarkers such as CRP, are proven to be a direct causal factor to worse prognosis in SICH, it could lay the foundation and act as a catalyst to more studies and to more targeted researches in the field of neuro-immunogy, that may shed a brighter light and generate new treatments in ICH patients. The aim of this study is such – to further reinforce the notion that elevated WBC and CRP levels do dictate a worse prognosis or outcome in patients with SICH.

3.2 Importance and Validity of Research

In this study, we aim to prove that elevated WBC and CRP levels could lead to worsened outcome and poor prognosis in patients presenting with SICH. It is our hope that the findings of this study would resonate with previous studies conducted by Di Napoli M et al. in 2011, where elevated CRP level was found to determine enlargement of haematoma and the consequent worsening of clinical outcome. Likewise this study would like to be in line with findings of research conducted by Wang J et al. in 2007 which implicated elevated WBC levels to worsened clinical outcome. We would like to remain optimistic that the supplemental evidence found in this study would generate clinical interest especially in the local setting in the field of neuro-immunology and also reinforce the understanding of the pathophysiology mechanisms involved in the natural history of SICH. At present, there has been no such study conducted in the local setting to examine the importance and relation of inflammatory biomarkers in the clinical pathology of SICH. Therefore, with a prospective study design, we intended to examine the correlation between elevated WBC and CRP with clinical outcome, in patients with SICH.

3.3 General Objectives

The mainstay purpose of this study is to evaluate the changes in peripheral leukocyte count (WBC count) and C-Reactive Protein (CRP) levels on admission and at 72 hours of admission in patients with SICH and to correlate these findings with survival and functional outcome at 6 months.

3.4 Specific Objectives

1. To assess the general demographic characteristics of patients presenting with spontaneous intracerebral haemorrhage
2. To determine the relationship of WBC count on admission and at 72 hours following admission , with overall 6 month survival
3. To determine the relationship of CRP level on admission and at 72 hours following admission , with overall 6 month survival
4. To determine the relationship of WBC count on admission and at 72 hours following admission , with 6 month clinical outcome based on Glasgow Outcome Score (GOS)
5. To determine the relationship of CRP level on admission and at 72 hours following admission , with 6 month clinical outcome based on Glasgow Outcome Score (GOS)

3.5 Research Questions

1. What are the general demographic characteristics of patients presenting with SICH?
2. Is there a significant relationship between WBC count on admission with 6 months survival?
3. Is there a significant relationship between WBC count at 72 hours of admission with 6 months survival?
4. Is there a significant relationship between CRP level on admission with 6 months survival?

5. Is there a significant relationship between CRP level at 72 hours of admission with 6 months survival?
6. Is there a significant relationship between WBC count on admission with 6 months outcome based on Glasgow Outcome Score (GOS)?
7. Is there a significant relationship between WBC count at 72 hours of admission with 6 months outcome based on Glasgow Outcome Score (GOS)?
8. Is there a significant relationship between CRP level on admission with 6 months outcome based on Glasgow Outcome Score (GOS)?
9. Is there a significant relationship between CRP level at 72 hours of admission with 6 months outcome based on Glasgow Outcome Score (GOS)?

3.6 Research Hypothesis

1. There is significant relationship between WBC count on admission with 6 month survival and clinical outcome based on Glasgow Outcome Score (GOS)
2. There is significant relationship between WBC count at 72 hours following admission with 6 month survival and clinical outcome based on Glasgow Outcome Score (GOS)
3. There is significant relationship between CRP level on admission with 6 month survival and clinical outcome based on Glasgow Outcome Score (GOS)
4. There is significant relationship between CRP level at 72 hours following admission with 6 month survival and clinical outcome based on Glasgow Outcome Score (GOS)

CHAPTER 4

MATERIALS AND METHODS

4.1 Research Design

This is a prospective, descriptive and correlational study conducted in a single centre to determine the relationship between WBC count and CRP level with 6 months outcome and survival, in patients admitted under Hospital Umum Sarawak for spontaneous intracerebral haemorrhage. Patients will be either admitted under Department of Neurosurgery or Department of Internal Medicine at Hospital Umum Sarawak (HUS) for further management of the intracerebral haemorrhage. The study was approved by the Malaysian Medical Research and Ethics Committee (MREC). [NMRR ID : 14-1711-21272 (IIR)]

4.2 Research Location and Duration

This study was conducted in a single tertiary centre – Hospital Umum Sarawak (HUS). Patients who fulfill the inclusion and exclusion criterias will be selected and included in this study. The total study duration was over a span of 2 years , from April 2013 till April 2015.

4.3 Inclusion criteria

The following are the inclusion criteria needed to be fulfilled by each patient prior being recruited for the study :

a) Any patients with spontaneous supratentorial intracerebral haemorrhage secondary to uncontrolled hypertension
b) Age 30-75 years old
c) Patients presenting with spontaneous intracerebral haemorrhage for the first time
d) Glasgow Coma Scale (GCS) score on admission between 9-14
e) Size of intracerebral haemorrhage ranging from 10 – 30 cm^3

4.4 Exclusion criteria

The following are the exclusion criteria for patients deemed not eligible to be included in the study.

a) Glasgow Coma Scale (GCS) score of less than 9/15
b) Patients presenting with spontaneous intracerebral haemorrhage other than due to hypertension
c) Patients with infatentorial bleed, brainstem bleed or thalamic bleed
d) Patients with incomplete study duration (minimum 6 months)
e) Pregnancy
f) Patients with culture positive infection on admission

g) Patient with underlying renal disease, hepatic disease, cancer or chronic systemic autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus etc.

h) Patients who were treated surgically

i) Patient who has undergone recent surgery or had major trauma (<4 weeks prior to SICH)

j) Patients with recurrent intracerebral haemorrhage

k) Patients with intraventricular bleed

l) Patients with recent ischemic heart disease event like myocardial infarction

m) Patients with acute or chronic infections (<4 weeks prior to SICH)

4.5 Method of research

In this prospective research, we aim to study the relationship between WBC count and CRP level on admission and at 72 hours, with patient's 6 month functional outcome (based on Glasgow Outcome Scale) and 6 month survival. We prospectively recruited all consenting patients fulfilling the inclusion and exclusion criterias, admitted to Hospital Umum Sarawak (HUS), with a diagnosis of SICH within 24 hours after onset of the stroke. The case subject of SICH was defined as abrupt onset of spontaneous intracerebral bleeding which was confirmed by cranial CT scan. Consents were obtained through their family members or legal representatives. Patients fulfilling the inclusion and exclusion criteria underwent detailed screening protocol which consisted of complete medical history, full neurological examination, blood tests which included routine Full Blood Count (FBC), CRP, renal and liver function tests, coagulation profile, ElectroCardioGram (ECG) and Chest X-ray. Data related to the age, sex, and general demographics related to patient was collected. GCS score and haematoma volume on cranial CT

was assessed. The estimation of the hematoma size and volume was calculated based on the Kothari's method of AxBxC/2 formula (as described earlier in Chapter 2). All routine biochemistry and hematology results were collected on admission and at 72 hours of admission .Blood that was collected for laboratory analysis was collected in EDTA and plain tubes. Firstly, the blood was collected in an Ethylenediaminetetraacetic acid (EDTA) containing tube and was sent for WBC level assessment and the blood which was collected in plain tube was sent for CRP level estimation.

WBC count was determined with an automated haematology analyzer (SYSMEX Haemato-analyzer) . CRP on the other hand, was determined via a qualitative method using latex agglutination technique – by using the AVITEX-CRP kit (provided by OMEGA Diagnostics). The AVITEX-CRP latex particles are coated with antibodies
to human CRP, as described by Singer et al. in 1957. When the latex suspension is mixed with serum containing elevated CRP levels on a slide, clear agglutination is seen within 2 minutes. Examine the test slide under a strong light source after 2 minutes. A positive result is indicated by the obvious agglutination of the latex, in a clear solution. A negative result is indicated by no change in the latex suspension on the test slide. AVITEX-CRP has a detection limit of 5 mg/l of CRP in the patient's serum. Positive results will be obtained at a CRP serum concentration above 5 mg/l and negative results will
be obtained at 5 mg/l and below. After baseline WBC counts and CRP concentrations on admission were obtained , patient was subsequently admitted to the ward for further management. Laboratory personnel were blinded to clinical information and quality control was maintained.